Remarks

The January 6, 2009 Office Action withdrew the prior objections/rejections, but formulated new obviousness rejections relying on Cosnier et al. with an additional piece of art, U.S. patent 5,575,895 ("Ikeda et al."). In view of the amendment above and arguments below, reconsideration is respectfully requested.

Nature Of Amendments

The independent claims, and thus all claims, have been amended to clarify that the main reagents (dehydrogenase enzyme, NAD^+ or $NADP^+$, NADH or NADPH reductase and redox active agent) are dissolved or dispersed in the buffered solution. This amendment is well supported by the specification. For example, amounts of each such reagent are described in terms of their molarity (see for example the passage spanning pages 5 and 6). Further, it is indicated at page 9, lines 29 to 30 that when the liquid enters the sensor, the enzymes and redox agent are "redissolved".

Summary Of Non-Obviousness Arguments

The Office Action asserts that Cosnier et al. discloses an electrochemical cell comprising a solution comprising, inter alia, a dehydrogenase enzyme and a reductase. It further asserts that the only difference between the disclosure of Cosnier et al. and the subject matter of, for example, claim 15 relates to the presence of a buffer.

Applicants respectfully disagree with this analysis since Cosnier et al. does not disclose a solution comprising a dehydrogenase enzyme and a reductase. Rather, these reagents are immobilized within a polymer matrix attached to the electrode (Fig. 2) and are not present in the solution itself.

A further difference between the subject matter of, for example, claim 15 and the disclosure of Cosnier et al. is accordingly that the reagents of the present claims are dissolved or dispersed in the solution, whereas those of Cosnier et al. (at least with regard to the dehydrogenase enzyme and reductase) are entrapped within a solid polymer matrix. In order to further highlight this difference, it has now been made more explicit in the claims that the reagents

present in the buffered solution are dissolved or dispersed.

As discussed in more detail below, neither Cosnier et al. nor Ikeda et al. teach or suggest to the skilled person a method of detecting or analyzing the amount of NADH or NADPH present in a sample using the main reagents which are all dissolved or dispersed in solution. The claimed subject matter is accordingly not obvious from Cosnier et al. and/or Ikeda et al.

Detailed Analysis Of Cosnier et al.

As is described in paragraphs 7 to 9 of the Wong declaration submitted with our response of 22 October 2008, Cosnier et al. describes an electrochemical system in which the reductase and dehydrogenase enzymes are immobilized within a polymer matrix attached to the electrode. Section 2.2 (see page 686) of Cosnier et al. describes the steps of adsorption of the materials onto the carbon electrode surface, followed by electropolymerization in order to generate the enzyme-containing polymer matrix. Figure 2 depicts the resulting electrode graphically. A polymer matrix is attached to the electrode surface and the dehydrogenase and flavin reductase are entrapped within this polymer matrix. The polymer contacts the solution to enable charge transport between the polymer layer and the solution, but the immobilized flavin reductase and dehydrogenase remain within the polymer matrix and do not move into a solution.

Claim 15 required an electrochemical cell comprising a buffered solution comprising the dehydrogenase enzyme and reductase. In any event, it is now more explicit in the claim that the dehydrogenase and reductase enzymes are dissolved or dispersed in the buffered solution.

As discussed above, the dehydrogenase and reductase enzymes of Cosnier et al. are entrapped within the polymer matrix and are not dissolved or dispersed within the solution. A major difference between Cosnier et al. and the subject matter of claim 15 is accordingly the presence of the reagents in solution, rather than immobilized to the electrode.

The use of these reagents in such a solution is not obvious from the teachings or suggestions of Cosnier et al.

It is well known in the art that the direct electrochemistry of NADH at electrodes is prone to a number of problems. These include the need for high overpotentials and the problems of adsorption of NADH and NAD^+ on the electrode surface. The issue of high overpotentials is a particular problem: the formal potential value for NADH is -0.561V vs SCE, however, a high overpotential of the order of 1V is required to oxidize NADH to NAD^+.

This overpotential means that a device which directly oxidizes NAD(P)H is prone to interference from common biological components. It has also been shown that this direct oxidation reaction poisons all common electrode surfaces (such as platinum, carbon and gold) very rapidly, leading to degradation in both the amount of NAD^+ produced and the current obtained.

Cosnier et al. itself highlights that the "major obstacle of the considerable overpotentials required for the electro-oxidation of NAD(P)H" in the first paragraph of the article. Cosnier et al. goes on to describe the problems of such high potentials, in particular interference from more easily oxidizable species and electrode fouling. The skilled reader would therefore understand from Cosnier et al. itself that a practically useful device based on the direct oxidation of NADH is not feasible.

Cosnier et al. further teaches that these problems may be overcome by immobilizing electroenzymatic mediators (i.e. at least reductase enzyme) onto the electrode surface. The remainder of the Cosnier et al. article focuses on the development of sensors having immobilized enzymes. The teaching of Cosnier et al. is therefore that immobilization of the electroenzymatic system is essential in order to make a useful biosensor.

Notwithstanding these contrary teachings, the present inventors have successfully coupled a reductase to the electrode surface via a redox couple (redox active agent) to enable to the oxidation of NAD(P)H with all reagents in solution. This has been achieved despite the problems of overpotentials and interference described in Cosnier et al.

This development is significant in a number of respects. In particular, the immobilized reagents of Cosnier et al. will suffer from poor charge transport within the immobilized polymer layer and poor accessibility or partitioning of the NAD(P)H/NAD(P)+ into or out of the immobilized layer. These factors can adversely affect both the accessible calibration range and the timescale of measurement. A system in which all reagents are dissolved or dispersed in solution, as is claimed in the present invention, leads to a much more effective and rapid charge transfer between the various species.

Thus, Cosnier et al. directly teaches away from biosensors in which all major reagents are dissolved or dispersed in solution, indicating that the overpotentials required present a major obstacle to biosensor development. In contrast, the skilled person would understand from Cosnier et al. that immobilization of the electroenzymatic reagents onto the electrode surface is essential in order to obtain a workable biosensor. The subject matter of claim 15 is therefore not obvious from Cosnier et al.

Detailed Analysis Of Ikeda et al.

Ikeda et al. describe an electrochemical biosensor. Particular sensors mentioned are fructose sensors (based on fructose dehydrogenase) and glucose sensors (based on glucose dehydrogenase). However, none of the electrochemical reactions described by Ikeda et al. involve the oxidation of NAD(P)H. The reactions described are therefore very different from those of Cosnier et al.

As discussed above, the particular motivation for immobilizing the enzymes reagents in Cosnier et al. is related to the problems of direct oxidation of NAD(P)H. The skilled person would not glean any further teaching as to how to carry out such NAD(P)H oxidation reactions from patents such as Ikeda et al. which relate to entirely different types of reaction. Therefore, the skilled person would not have been motivated to consider the teaching of Ikeda et al. when working on the oxidation of NAD(P)H.

In this regard, the electrochemical cells of Ikeda et al. include a carboxymethyl cellulose layer onto which a reaction

layer (including the enzyme reagent fructose dehydrogenase) is formed (see example 1, column 5, lines 17 onwards). The reagents are not therefore entrapped within an electropolymerized layer in the same manner as described by Cosnier et al. However, since Ikeda et al. do not describe NAD(P)H oxidation reactions, the skilled person would not be motivated to employ the techniques of Ikeda et al. in producing an electrochemical cell for NADH oxidation. In particular, nothing in Ikeda et al. would suggest to the skilled person that NADH oxidation would be feasible without immobilizing the enzyme reagents within a polymer matrix. The claimed subject matter is therefore not obvious from the combination of Cosnier et al. and Ikeda et al.

Further evidence for the non obviousness of the claimed subject matter can be gleaned from the large amount of work which has been carried out in the field of NAD(P)H oxidation over the last 20 years, without the present invention being made. Throughout the 1990s one of the largest research areas in the field of bioelectrochemical sensing was the production of dehydrogenase based biosensors. Since NAD(P)H is a cofactor in these reactions, much work has been carried out involving the reversible electron transfer of NAD(P)H either directly with the electrode or via a mediator. However, despite this focus on the oxidation of NAD(P)H, the electrochemical reaction using a dehydrogenase, $NADH^{+}$ or $NADP^{+}$, a reductase and redox active agent, all of which are present in solution, has not previously been proposed. That such techniques have not previously been suggested despite the large amount of work which has been carried out in the area, in the face of a long felt need, further evidences the non obviousness of the present invention.

Other Claims

Claims 3, 4, 6 to 14, 16 and 17 directly or through dependency now contain the same distinguishing feature as now added to claim 15, namely the presence of a buffered solution wherein the reagents are dissolved or dispersed in the solution. These claims are accordingly also non-obvious over Cosnier et al. and Ikeda et al. for the same reasons as set

out above.

Claims 18 and 19 relate to an electrochemical cell wherein a solution containing the reagents dissolved or dispersed therein has been dried. This dried reagent mixture will redissolve on contact with the sample (see page 9, line 29 onwards). It is therefore not an immobilized system.

In contrast to the reagents in the electrochemical cells of claims 18 and 19, the reductase and dehydrogenase enzymes of Cosnier et al. are entrapped within a polymer matrix such that they remain within the matrix even on contact with a solution, i.e. they are immobilized. A difference between the disclosure of Cosnier et al. and the subject matter of claims 18 and 19 is therefore that the reductase and dehydrogenase enzymes of Cosnier et al. remain immobilized in the polymer matrix on contact with a liquid sample, whereas the mixture of reagents in the cell of claims 18 and 19 will become dissolved or dispersed in the sample on contact.

As discussed above, Cosnier et al. teaches that a workable sensor can only be produced where the enzymatic reagents are immobilized. The skilled person would therefore understand from the teaching of Cosnier et al. that a workable sensor would not be produced where the reagents would dissolve or disperse in the sample on contact of the reagents with the sample. Thus, for reasons analogous to those discussed above, Cosnier et al. teaches away from an electrochemical cell having dried reagents as claimed in claims 18 and 19.

Conclusion

As such, reconsideration and allowance are respectfully requested of claims 3, 4 and 6-19, as amended. No additional fee is believed necessary for consideration of this amendment. However, if one is, please charge it to Deposit Account 17-0055.

Respectfully submitted,

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